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Plant Parasitic Nematodes: A Major Constraint in Fruit Production

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Abstract

The plant parasitic nematodes are one of the major limiting factors in fruit trees specially in citrus, banana, papaya, jackfruit, guava etc. The root knot nematodes are the major problem amongst all those nematodes infecting on these trees. Besides, directly causing a huge losses, they are also inviting the secondary plant pathogens, like fungi, bacteria, viruses etc. amongst which, the wilt fungus, *Fusarium* species increase the severity of the diseases. This complex disease is becoming much severe in banana and guava recent years. In citrus also, the citrus nematodes, *Tylenchulus semipenetrans*, is causing havoc by slow decline disease and it is becoming a major problem in horticultural nurseries because these nurseries are a hot spot of citrus nematodes. So, unknowingly these nematodes get spread to different places. The management of these nematodes by simple, cheap and eco friendly methods, is very important as it will decrease the monetary pressure on cultivators as well as it helps in improving environmental pollution.

Keywords: plant parasitic nematodes, fruits, *Meloidogyne* spp., *Tylenchulus semipenetrans*, *Pratylenchus* spp.

1. Introduction

Plant parasitic nematodes cause considerable economic losses in fruit crops. The main loss is the destruction of roots which hinders the movement of nutrients and water through the vascular system, so, there is drastic reduction in fruit or bunch weights, the quality of fruits is deteriorated and there is a drastic reduction in plant numbers. Furthermore, roots damaged by nematodes are easy prey to fungi and bacteria which invade the infected roots and feed on them and thus roots decay rapidly. The root-knot nematode, *Meloidogyne incognita*; the burrowing nematode, *Radopholus similis* and citrus nematode, *Tylenchulus semipenetrans* are the major nematode pests that infect these fruit crops. Around 30–40% loss in yield is due to these nematodes. The nematode infestation in fruit crops not only aggravate disease complexes but also breaks down disease resistance in certain varieties of fruit crops. These nematodes mainly spread through infested planting material to other uninfested sites. For an instance, in banana, paring or trimming of suckers is carried out before planting which is usually not adequate to eliminate deep infections in the suckers. The residual nematode population builds up and disseminates when they enter the irrigation system. Other routes of their dispersal include soil adhering to tractor tyres, shoes of labour and tillage implements. The major fruit crops which suffer severe nematode infestation, are discussed here.

2. Citrus

Citrus is grown in more than 125 countries in a belt between 35° latitude north or south of the equator. The citrus is generally consumed as fresh fruit, approximately 68% of the world's citrus production, and in international trade, about 11% of total production is used [1]. *Citrus* spp. are naturally deep-rooted plants [2, 3], and optimum growth requires deep, well-drained soils. The first nematode discovered in citrus, was, *Meloidogyne* sp. which parasitized the citrus in Florida in 1889 but people were unaware about these nematodes. Again in 1913, Thomas discovered the citrus nematode infecting citrus in California. Nathan Cobb had reported it as a new species, *T. semipenetrans*, and this was the causal agent of mottling disease in citrus in California, later identified as 'slow decline' because the trees declined in vigour but very slowly about in 10–15 years. *T. semipenetrans* has been found in every citrus-growing region of the world since its discovery [4]. Field infestations within United States, infect 50–90% of citrus orchards in Arizona, California, Florida, and Texas, as well as local vineyards in California [4–6]. The major economic nematodes causing diseases in citrus, are *T. semipenetrans*, *Pratylenchus* spp. and *Meloidogyne* spp.

2.1 Citrus nematode (*T. semipenetrans*)

In world, Cobb in [7] first described its distribution, morphology and life history. In India, it was first reported by Siddiqi [8] at Aligarh, Uttar Pradesh. About 80% of the citrus trees were reported to be infected with this nematode. This slow decline further results in 'die back' disease in most of the citrus trees in India and is a major problem that is estimated at 8.7–12.2%. The citrus nematodes are found highly in number when the citrus orchards are established in the soil which is finely-textured or sandy having high organic matter content. Nematode reproduction was positively favoured when there are fluctuations in soil salinity from high to low, while sandy soils poor in organic matter, hinder population increase [9]. In Florida and California (USA), there is 24–60% of *T. semipenetrans* infection in citrus orchards while it is 70–90% in commercial orchards of Brazil and Spain [10]. This shows that the citrus nematodes can infect under extreme range of environmental conditions. Unfortunately, infected nursery seedlings which are the main infection material transported from one to another, are not easily detected by the personnel involved in that business just because of unawareness [10]. If the infection is not severe, the roots show only lesions but, during severe infection, the sloughing of root cortex appears and roots die finally. Nematode infection increases the levels of the cell-damaging enzymes [11].

2.2 Symptoms

1. Reduction in vigour of the plant
2. Reduction in tree growth
3. Reduction in fruit number
4. Reduction in fruit size
5. Decline is from upper side to the lower side of the plant, so, called 'die back'
6. If the initial population is high, the death of plants occurs in very early age

7. The roots became sticky because of gelatinous matrix secreted by the females and soil particles glued to the roots which do not go after washing with water
8. The cortex separates easily if there is heavy infection because the females are feeding semiendoparasitically on the roots having their posterior two third of body part outside the root where they lay eggs and excrete the gelatinous matrix to protect the eggs
9. Leaves become smaller and chlorotic
10. Leaves fall due to poor vigour
11. If there is water stress during infestation, then wilting occurs
12. There is poor root development
13. The feeder roots decay very fast
14. Root death occurs due to heavy infestation
15. The diseased trees become dwarf and yield less than the healthy ones
16. Duncan [4] reported that the symptoms like reduced leaf and fruit size, canopy thinning, and die back of upper branches, are the most conspicuous symptoms of slow decline and that result in less yield

2.3 Life cycle

T. semipenetrans exhibits sexual dimorphism, i.e., different shape of male and female individuals at both the juvenile and adult stage. The life cycle duration is of 6–8 weeks from egg to egg [12]. The *T. semipenetrans* biology and ecology have been extensively studied [10]. The egg hatching takes place in 12–14 days at 24°C. The male larvae after second stage, do not feed and become mature in 7 days whereas the female takes 14 days to find the feeding site on the root and start feeding and moulting. The female juveniles can survive more than 2 years in the absence of roots [13]. The life cycle was of 14 weeks on *Poncirus trifoliata*, 10 weeks on *Ruta bracteosa* and 7 weeks on *Citrus aurantium* and *C. limettoides* [14]. The mature males are vermiform and mobile found in the soil or in the egg masses. Therefore, the feeding apparatus (stylet and oesophagus) of adult males is poorly developed and may be difficult to observe. *T. semipenetrans* is a sexually reproducing species that can occasionally reproduce by facultative parthenogenesis without the need of males. The mature females and their eggs are found attached to roots which are protected by soil particles that sticks to gelatinous matrix. The females are swollen and enlarged posteriorly often protruding on the root surface in a finger like protrusions while elongated and not swollen anteriorly generally embedded and hidden in cortical parenchyma. After hatching at optimum temperature, i.e., at 25°C, females lay eggs after 6 weeks, on the root surface in a gelatinous egg mass secreted from the excretory pore.

2.4 Histopathology

The second stage larvae enter the root surface and start feeding on the mature part. After moulting, the immature females penetrate deeper in the cortex region

and their neck becomes longer to feed inside. The posterior portion remains outside of the root. They establish a feeding site around their stylet where the cortical cells change into food sink by reaction of dorsal oesophageal gland secretions. These are called 'nurse cells' which provide food to the developing females. The nurse cells are thick-walled cells with modified cell organelles like enlarged nucleus and nucleolus. These cells have no vacuoles. The cells are gradually destroyed by their feeding and hence the plants can not draw food and water for their growth, so change in development proceeds which finally results in poor vigour.

2.5 Host range

Unlike many nematodes, *T. semipenetrans* has a restricted host range. Many plants belonging to Rutaceae family, were found as hosts of this nematode. It was reported that from 23 countries, 29 species of *Citrus*, 21 citrus hybrids and 11 other species as the hosts [15]. Except these plants, it was also reported to attack on other plants like *Andropogon rhizomatus*, *Panicum* spp., Olive (*Olea* spp.), grapevines (*Vitis* spp.), Persimmon (*Diospyros lotus*), Pear (*Pyrus communis*), *Calodendrum capense*, climbing hemp weed (*Mikania batatifolia*) and Lilac (*Syringa vulgaris*) [16–19]. Parvatha Reddy and Singh [20] reported that the citrus nematode also attacked grapes and loquats. It can parasitize more than 75 plant species belonging to rutaceous species (especially citrus and their close relatives) which are its suitable hosts [13]. Till now, there have been no reports of *T. semipenetrans* infecting herbaceous plants [17]. El-Mohamedy et al. [21] reported numerous citrus varieties from Egypt, Washington Navel, Valencia orange, Mandarins group varieties (*C. reticulata*), lemon (*C. aurantifolia*), and Balady orange (*C. sinensis*), Grapefruit (*C. × paradisi*), Sour orange (*C. aurantium*), and Kumquat (*C. japonica*) infected with this nematode [22, 23].

2.6 Complex disease

The citrus nematode also interacts with other plant pathogens and increase the severity of the disease. The wilt fungus, *Fusarium oxysporum* and *F. solani* with citrus nematode caused the death of citrus trees. The interaction between *T. semipenetrans* with such microorganisms occurs in inconsistent ways. It can reduce the infection of roots by *Phytophthora nicotianae* after the infection to citrus seedlings and it can also increase the virulence of *Fusarium solani* [10]. It was reported that the high population level of nematodes when interacted with *F. semitectum*, got synergistic effect on the infected citrus seedlings [24]. *Fusarium* spp. can be pathogenic on citrus roots alone [25] or in combination with nematodes [26], which leads to the great destruction of the feeder roots. The loss of feeder roots due to feeding of nematodes results in increase of drought stress and decrease of soil nutrient uptake, leading to chlorosis and loss of leaves. Affected trees do not die, but have an unthrifty appearance and yield fewer, smaller fruits than uninfested trees.

2.7 Nematode spread

The major cause for the spread of this nematode is because of distribution of infested planting material from the horticultural nurseries. Once the infested soil is taken with the planting material to distant places, it will spread this nematode to new sites. The other spreading agents are human and animals with the infested soil on their feet, agricultural implements, and water. They can survive in the soil for long periods in the absence of host that enables them to infect after a long time also. The main source of infection in the citrus plant, are, infected seedlings, organic

fertilizers, plant materials, irrigation, and machinery which are affecting growth and yield in the newly planted area [27]. In Egypt, which is a highly ranked citrus producing country [27], the citrus orchards were incorporated with the soil brought from silty soil from the Nile Valley for mulching and improving the soil quality but that soil was nematode infested, so the disease incidence got aggravated [28]. So, with time, the nematode spread their populations in that soil and the losses increased [27]. Soil moisture is often inversely related to population growth of *T. semipenetrans* [29–31].

2.8 Management

2.8.1 Preventive measures

The most important and effective method is to take every effort to avoid the use of infested planting materials and contaminated farm implements when new plantations have to be established. In the orchard, proper drainage and light should be there and shade should be avoided as far as possible. New nurseries should not be established near the old citrus orchards. All sanitation practices should be taken to avoid nematode infestations. Use of certified nematode-free material for planting, is also very important. If there is established infection, the citrus orchard should be rotated with annual crops for 1–3 years before replanting helps to reduce citrus nematode populations. For intercropping, Marigold is an excellent crop which has repellent action and reduces the population of nematodes in citrus [32].

2.8.2 Biological measures

Application of *Pseudomonas fluorescens* @ 20 g/tree. *Paecilomyces lilacinus* parasitize nematode eggs and females, reduces the number of plant parasitic nematodes in soil, *T. semipenetrans*. Park et al. [33] reported that *P. lilacinus* could produce leucino toxin and other nematicidal compounds. *Trichoderma* spp. play major roles in controlling plant diseases in roots and soil. The *Trichoderma* spp. have antagonistic activities to be used as effective biological control agents for many plant diseases which are caused by soil borne fungi and nematodes [34]. Although *Bacillus subtilis* was reported as a bio-agent against soil borne fungi [35] some strains of *B. subtilis* exhibited enormous potential as bioagent in the management of nematodes [36]. *B. subtilis* produces antibiotics as bacterocin and subitisin [37, 38]. Streptomycetes are the major group of actinomycetes producing secondary metabolites that could decrease the invasive juveniles of root-knot nematodes. Streptomyces is known for its chitinolytic activity which produces more extracellular chitinase [39]. Some species of streptomycetes release compounds like antibiotic that inhibit the growth of plant-pathogenic fungi [40] and plant parasitic nematodes [41]. Qingfei et al. stated that *Streptomyces* spp. produce lytic enzymes and nematicidal compounds and can be one of candidates for bio-agents against nematodes. Le Roux et al. [42] demonstrated that *P. lilacinum* individually controlled *T. semipenetrans* Cobb on mandarin and rough lemon effectively, but when the fungus was combined with oil-cakes, the results were more significant.

2.8.3 Chemical measures

In heavy infestation, many nematicides have successfully been used to lower down the population of *T. semipenetrans* on citrus in many locations. The soil treatments with soil solarization and nematicides is highly beneficial in both replanting conditions and already established orchards. Pre-plant application of carbofuran 3 G @

100 g/tree, was found highly beneficial. The nematicide application often increases the citrus yield [10]. Nemastop (natural oils) as commercial nematicide, play very important role in controlling nematodes. The effect of Nemastop on the nematode might be due to alkyl cysteine sulfoxides which released a mixture of volatile alkyl thiols and sulphides [43]. Whereas, Nemaphos belonging to organophosphate group [44] showed a highly performance systemic nematicide. When halogenated hydrocarbons are used as pre-plant soil fumigants, these can effectively control *T. semipenetrans* for many years [45–48]. However, to maintain the low population and higher yield, one has to apply the chemicals repeatedly. In the first year of treatment, the effect will be little on yield and fruit quality but the efficacy to increase the growth and yield parameters can be observed in the following year [49–51].

Oxime carbamates (aldicarb, oxamyl, Carbofuran) and organophosphates (fenamiphos, ethoprophos and cadusaphos) are the two main groups of nematicides which are available in the market for the management of citrus nematode. Of these, granular formulation of Cadusaphos has shown greater efficacy against the *T. semipenetrans* [52–55]. Irrigation is generally recommended before nematicide application for better results.

2.8.4 Resistant rootstocks

The use of resistant root stock is the best method to avoid the disease if available. It was reported by many workers that the use of resistant (Swingle citrumelo) rootstocks and certified propagative material which are free from nematode parasites of citrus, are promising cultivars for preventing the loss caused by *T. semipenetrans* to citrus [56–58]. In Florida, this approach has significantly reduced the spread of this parasite, making the land free from nematode infestations [59]. In California vineyards, resistant (Ramsey) or moderately resistant (vinfera Dog Ridge) grape rootstocks were used successfully [60] (McKenry, personal communication). To get sustainable agriculture, planting of nematode certified citrus and grape rootstocks, is an excellent practice that should be adopted for other fruit crops also which are susceptible to nematode infections. Resistance-breaking biotypes were developed on Swingle citrumelo [61]. The commercially resistant rootstock, Swingle citrumelo is common in Florida and combined with regulation program of the citrus nematode, has decreased the spread of *T. semipenetrans* dramatically [62]. Using a resistant rootstock is recommended whether or not nematodes are present. Trifoliolate orange is known to be tolerant to citrus nematode.

2.8.5 Soil solarization

Soil solarization is an effective method to disinfest the upper soil layers by moistening the soil and covering with a clear plastic sheet in regions with hot and dry summer months. This method is highly beneficial to manage the population of insects, soil borne pathogens, weed seeds and nematodes by altering the physical, chemical, and biological properties of the soil. In South Africa, solarization has not shown promising and there was inconsistent suppression of the citrus nematode and tree growth [63], which may be because these nematodes are found deep within the soil profile and so, are not affected by solarization that is most effective for the upper soil layers [64].

2.8.6 Steam treatment

Steam treatment of soil is widely used for the control of nematodes in planting material and is shown very effective. In this method, the soil is heated up to 70°C, mainly by means of aerated steam. It is very useful and economical for disinfestation

of nursery beds. Steam treatment of vermiculite or tuff stones is usually effective but is more difficult for peat soils due to their high water content [65]. The dipping of planting material in hot water is also effective but here the temperature of water should be taken care of, otherwise the germination may get affected. Bare root dipping of citrus seedling in hot water at 45°C for 25 min [66] or 50°C for 10–20 min [67] was found to be effective without any adverse effect on the germination.

2.9 Lesion nematodes (*Pratylenchus* spp.)

There are three species of *Pratylenchus* which can affect citrus i.e., *P. coffeae*, *P. brachyurus*, and *P. vulnus*. All are reported from Egyptian citrus orchards [68]. The most pathogenic species is *P. coffeae* [10]. Lesion nematodes, being a migratory endoparasites, cause infection mainly in the feeder roots during their movement by penetrating the cortical tissue, but they do not invade the vascular tissues. After their infection, the secondary organisms infect the root tissues and then the vascular tissues also got infected. *P. coffeae* is obligatorily amphimictic, all stages infective with males feeding in the roots [69]. Its reproduction is highest at high (26–30°C) soil temperatures. At those temperatures, the life cycle is completed in less than a month, and it can achieve densities of up to 10,000 nematodes/g root [70] and persist in soil roots for at least 4 months. This leads to root weight reduction by half and growth reduction ranging from 49 to 80% in young trees in field conditions. A 3-fold to 20-fold differences between infected and non-infected trees was observed in terms of the numbers of fruit [71]. Commercial rootstocks resistant to *P. coffeae* are yet to be identified. A lesion nematode, *P. coffeae*, was detected on citrus in Sao Paulo State, Brazil and found to infest about 1% of the nurseries and orchards [72].

The biology of *P. brachyurus* is similar to that of *P. coffeae*. [13]. It has been established as a pathogen of citrus seedlings across several soil types. [73]. After controlling *P. brachyurus* with aldicarb, yields of Valencia orange trees grafted on rough lemon were increased, and plants sustained reduced frost damage in the winter [51]. Some studies failed to note the fact that *P. vulnus* has been found associated with citrus plants in Egypt [10, 13, 68]. This species is capable of causing significant damage to citrus seedlings but has not been reported to damage mature plants [74]. Biology, population growth rates, and root damage are similar to those described for *P. coffeae* [13]. Several *Pratylenchus* species have been identified in Egyptian citrus orchards based on field studies [68].

Host range: *Citrus limon*, *C. sinensis*, *C. reticulata*, banana [75] and *Citrus jambhiri* [76].

2.9.1 Management

Chemical: Fensulfotion or phenamiphos @ 4.4 kg a. i./ha and aldicarb or carbofuran @ 4 kg a. i./ha [77].

Resistant root stocks: Trifoliate orange (*Poncirus trifoliata*), Rubiboux 70-A5, hybrids of *Microcitrus australis* x *M. australasica*.

2.9.2 Root knot nematodes (*Meloidogyne* spp.)

Root knot nematodes attacking citrus trees, are not reported much and is little in distribution [10]. Only a few locations in world, have been found to be infested with this nematode. A pathogenic root knot nematode species (known as Asiatic pyroid citrus nematode) recorded from Taiwan and New Delhi could cause elongated galls on citrus roots [13]. It can complete its life cycle on several citrus and other plant species. The common species are *Meloidogyne incognita*, *M. javanica*, and *M.*

arenaria reported to infect roots of Troyer citrange and sour orange, causing small galls, but their multiplication is not recorded [78]. *M. indica* was reported from citrus tree at some locations in India [79].

2.9.3 Symptoms

1. Severe stunting of the plants
2. Yellowing of the leaves
3. Plants show unthriftness
4. Symptoms of twig blight appear
5. Slow drying of the tree
6. Small to large galls on the roots are the characteristic symptoms
7. Poorly developed root system [80]
8. The trees do not flower and fruit
9. Large cavities are found on the roots
10. Egg masses float on the root surface

2.9.4 Management

Chemical methods: Seedling dip treatment with carbosulfan @ 500 ppm for 6 h can effectively control root knot nematodes.

Organic amendments: Mustard cake, farm yard manure and poultry manure @ 2.5 kg/plant were found effective against root knot nematode and increasing the plant growth.

Host resistance: Resistant rootstocks, like, Rangpur lime 8784, Sour orange Tirupati, Citrumello 4479, Rangpur 8748, Rangpur lime chethalli, Trifoliate orange chethalli, Nasaran, Hazara Australia, Rangpur lime Kirumakki, Pramalini and Anand Selection were moderately resistant.

3. Guava

The common guava (*Psidium guajava* L.) is indigenous to tropical America. It is a popular fruit generally consumed as fresh fruit but also processed as jam, paste, puree, canned shells and juice for round the year use. It is grown throughout the tropics and subtropics and is of commercial importance in more than 60 countries [81]. This fruit tree also suffers many nematode infections. Mostly root knot nematodes were reported from guava roots and after infection, the roots are predisposed to wilt fungus which is common wilt disease causing pathogen found in guava. This interaction resulted increased disease severity. Khan et al. [82] observed greater damage to guava with both *Helicotylenchus dihystera* and *Fusarium oxysporum* than with the nematode alone. In India, *Hoplolaimus indicus* was found to be a pathogen of guava [83, 84] and *Tylenchorhynchus cylindricus*, in numbers of up to 2000 nematodes/100 cm³ of soil, was found associated with damaged guava trees in Iran [85]. In the past two decades, the

root-knot nematodes, *Meloidogyne* spp. Göldi, have been reported on some species of the tropical fruit trees grown in the region [86]. Gomes et al. [87] demonstrated that guava trees infected with *M. mayaguensis* had deficiencies in nitrogen, potassium, phosphorus, calcium, and magnesium, and that these mineral deficiencies were proportionally related to the severity of root galling and root decay, which eventually led to the death of guava trees within a few months. Recently a new species, *M. enterolobii* has been found to be widely associated with many guava trees.

3.1 Symptoms

1. Yellowing of trees
2. Leaves shed prematurely
3. The branches start drying
4. The trees show less vigour
5. The trees fruit very less
6. The affected trees dry within three months
7. Multiple small galls can be seen on the roots
8. The affected roots show necrosis
9. There is decrease of feeder roots [88]

3.2 Spread

In guava, grafts are often produced in polythene bags with a substrate mixture (sand + soil + FYM or other organic manure). In most cases the substrate mixtures harbour the aforementioned nematodes as well as other harmful fungi and bacteria. Generally, nurserymen do not treat the soil combination used in the production of fruit seedlings or grafts in their nurseries. As a reason, before applying the substrate, it should be treated with biopesticides.

3.3 Management

3.3.1 Prevention

Nematode free plants should be used for new planting and the orchard should be planted in nematode free soil. The soil used for the preparation of new plants should be sterilized. The equipment should be sterilised before using.

3.3.2 Deep summer ploughing

The soil for the planting of new guava plants, should be deeply ploughed during hot summer months. It should be repeated twice or thrice at 15 days interval.

3.3.3 Biological method

For biological control, many fungal and bacterial bioagents are used for the management of nematodes. The fungal bioagents, *Purpureocillium lilacinum* and

Verticillium clamydosporium were found as the most potent fungal parasites that can effectively control *Meloidogyne* spp. on many host plants [89]. Another fungal bioagent, *Trichoderma harzianum* effectively suppressed the population of the root-knot nematode, *M. enterolobii* in both soil and roots of guava in Thailand [90]. In Saudi Arabia, Al-Hazmi et al. [91] found a heavy colonization of the cysts of *Heterodera avenae* Woll. with the fungus, *Verticillium clamydosporium*. Rao [92] who reported that the fungus, *P. lilacinum* and the bacteria, *P. fluorescens* enriched the farm yard manure, fairly controlled the reniform nematode, *R. reniformis* and the root knot nematodes.

3.3.4 Organic and inorganic nitrogenous amendments

These amendments are useful both for managing plant parasitic nematodes as well as to improve the soil fertility [93]. At rates as low as 300–400 mg/kg soil, urea and ammonia were found to be effective in controlling plant parasitic nematodes [94]. Guava decline disease, a disease complex caused by *M. mayaguensis* in association with the fungus, *Fusarium solani*, has been successfully managed in a commercial guava plantations, with significant yield gains obtained by the use of cow manure and poultry compost [95]. Urea and nitrogenous fertilizer are considered to be good nematicides when applied at levels as low as 300–400 mg/kg soil because due to root infections, the roots become unable to take the minerals and nutrients [94, 96–98]. Previously it was also reported that organic and inorganic nitrogen amendments had a nematicidal effect against plant parasitic nematodes [93, 94, 99]. Gomes et al. [95] reported that the guava canopy when treated with organic soil amendments, particularly poultry compost and cow manure, gave better control of the root knot nematode, *M. mayaguensis*. Organic soil amendments not only promote the growth of soil microorganisms that are antagonistic to plant parasitic nematodes but also release specific toxic compounds during their decomposition that may have nematicidal effects against nematodes [99]. These organic amendments also improve crop nutrition and growth which lead to increased tolerance of plants against plant-parasitic nematodes [100].

3.4 Production of healthy rootstocks/grafts

Enriched substrates should be used for producing healthy grafts or rootstocks of fruit crops. A mixture of enriched FYM can be produced by mixing one ton of soil mixture consisting red soil and sand in equal proportions with 500 kg of FYM which is added with 2 kg each of *P. fluorescens* 1% W. P., *Trichoderma harzianum* 1% W. P., *Paecilomyces lilacinus* 1% W.P. + 50 kg neem or pongamia cake +5 kg carbofuran or phorate.

3.5 Spraying or drenching the nursery seedlings or grafts with bio-pesticides

The nursery seedlings or grafts can be treated by dissolving 5 g or 5 ml/l of water once in 10 days.

3.6 Management of nematodes in the main field

3.6.1 Soil application

Before planting the saplings of guava, the land should be thoroughly ploughed and soil should be brought to fine tilth. Then recommended doses of fertilisers

should be added because the vigour plants resist the nematode attack. Now carbofuran or phorate @ 20 kg–25 kg + 200 g neem/pongamia/mahua cake per acre should be added to reduce the initial population of nematodes. The optimum moisture should be maintained in the beds which is necessary for proper decomposition of organic matter. In case of organic farming, during the land preparation, application of two tons of FYM or 500 kg of neem cake/pongamia cake or one ton of enriched vermicompost with *P. fluorescens* + *Trichoderma harzianum* + *Paecilomyces lilacinus*.

3.6.2 Process of enrichment of FYM

For enrichment of FYM, 2 kg each of *P. fluorescens*, *Trichoderma harzianum* and *Paecilomyces lilacinus* formulation should be mixed with one ton of well decomposed FYM under shade and covered with mulch. An optimum of 25–30% moisture should be maintained for a period of 15 days. This mixture should be thoroughly mixed once in a week to promote maximum multiplication along with even growth of the microorganisms in the entire lot of FYM.

3.6.3 Process of enrichment of neem cake

Neem cake can be enriched by mixing with 2 kg each of *P. fluorescens*, *Trichoderma harzianum* and *Paecilomyces lilacinus* with one ton of neem cake under shade and covered with mulch. For next 15 days, an optimum moisture of 25–30% has to be maintained followed by thorough mixing once in a week to ensure maximum multiplication & uniform growth of the microorganisms in the entire lot of neem cake.

3.6.4 Process of enrichment of vermicompost

Similarly, vermicompost can be enriched by mixing with 2 kg each of *P. fluorescens*, *Trichoderma harzianum* and *Paecilomyces lilacinus* with one ton of vermicompost under shade and covered with mulch. For next 15 days, an optimum moisture of 25–30% has to be maintained followed by thorough mixing once in a week to ensure maximum multiplication & even spread of the microorganisms in the entire lot of vermicompost.

3.6.5 Application of bio-pesticides at the time of planting

At the time of planting, application of bio-pesticide enriched FYM @ 3 kg or enriched neem cake @ 250 g or enriched vermicompost @ 500 g/plant at an interval of six months.

3.6.6 Spraying

Spraying plants with organic formulation containing *P. fluorescens* & *Trichoderma harzianum* at regular 30-day intervals at a dosage of 5 g/l or 5 ml/l.

3.6.7 Drenching or application through drip irrigation system

Drenching of the above biopesticide @ 5 g/l or 5 ml/l at regular interval of 30 days.

4. Banana

It is also one of the important fruit crops in India, cultivated over 0.83 million ha, constituting about 44.3% of total fruit production. Generally, the farmers and

cultivators grow banana as cash crop and do the intensive cultivation on a commercial scale and as monoculture in the same field. This system invited several pests and diseases to this crop. Among these major biotic stresses, plant parasitic nematodes are causing severe losses in banana production not only individually but also with the interaction of wilt fungi, *Fusarium oxysporum* f. sp. *cubense* [101]. The major nematode causing economic yield loss globally, is the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949. Other nematode species that are found in banana cultivation along with *R. similis*, are, lesion nematode (*Pratylenchus coffeae*), spiral nematode (*Helicotylenchus multicinctus*) and *Meloidogyne* spp. In South Africa, 34 plant parasitic nematode species have been found associated with banana [102] (SAPPNS1). Likewise, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 are most commonly reported root knot nematode species found in association with local banana cultivars in Zimbabwe [103]. Root knot nematodes were the most abundant and together with spiral nematodes constituted 72% of the total plant parasitic nematode complex [104]. The root knot nematodes become larger in size in banana root tissue as the root tissues are thicker. These nematodes attack root and corm tissues causing damage which results in long vegetative growth cycle, late fruiting, production of small bunches, less fruiting and finally toppling of the plants. The spiral nematodes produce root lesions in the corm which attract the secondary pathogen, fungi and bacteria, that aggravate the damage of the root system [105]. Root knot nematodes are present in almost all banana plantations [106]. The general perception is that these nematode pests can cause severe damage to young plants, resulting in suboptimal growth and yield.

4.1 Host range

Both *M. incognita* and *M. javanica* has a wide host range of cultivated crops including most broad leaf weed species. To effectively control root-knot nematodes in bananas, special attention should be paid to weeding during fallowing or crop selection for rotation [107].

4.2 Losses

The diseases in banana are caused by 3–4 nematodes so, the quantification of the damage by individual species is, therefore, not possible. Willers [108] estimated that these pests caused a direct loss of 19% in total production of the crop.

4.3 Symptoms caused by burrowing nematode, *R. similis*

The origin of burrowing nematode is believed to be from Australia and New Zealand [109] from where recently new species have been described. The relative increase in worldwide distribution is mainly due to transfer of infected planting material domestically as well as internationally. The importation of banana cultivar Cavendish which is susceptible to nematode attack is often correlated with the wide distribution of *R. similis*. It is assumed that *R. similis* was introduced in Latin America and the Caribbean, on the cv. Gros Michel where it subsequently infested the more susceptible Cavendish cultivar [110]. In a study, 55 out of 57 burrowing nematode isolates, collected from Australia, Cameroon, Central America, Cuba, Dominican Republic, Florida, Guadeloupe, Hawaii, Nigeria, Honduras, Indonesia, Ivory Coast, Puerto Rico, SA and Uganda were morphologically similar to *R. similis*.

The following are the symptoms:

- Dwarfing of plants
- The size and number of leaves are reduced and bunch weight is also reduced.
- Dark red lesions appear on roots which is the identification of fungal infection after nematodes.
- The whole plant topples down in windstorms or heavy rains due to less vigorous roots after infection of plants.
- Heavily infected plants have thin pseudostems, while the leaves are yellowish or discoloured, greenish yellow bands appeared along the leaf blades [111].
- Infected plants are also prone to wilting during hot days because the plants could not take water due to root damage.

4.3.1 Management

- Field fallowing for a period of six months or longer should be done to avoid the continuity of the nematode generation.
- Crop rotation with non-host crops should be done.
- Use of disease-free planting material.
- Storage of large corms in the sun for two weeks prior to planting to kill the nematodes and their juveniles.
- Use of cover crop calapogonium is very useful to avoid the population.
- Removal of infested portions of corm before planting.
- Hot water treatment at 55°C for 15–25 min and the time and temperature limit should be strictly followed.
- Paring and Pralinage are important practice to take care the plants after infection
- Carbofuran 3 g @ 40 g/plant at 90 days after planting as this is systemic nematicide.
- Application of neem cake also enhances the plant growth as well as the management of nematodes
- Host range: Grapevine, Papaya, Pomegranate, Banana

Symptoms caused by Lesion nematode, *Pratylenchus coffeae*

- Extensive black or purple necrosis on roots, are found
- Stunting of plants as a characteristic symptom

- Reduction in size and number of leaves and in bunch weight.

Symptoms caused by Root knot nematodes, *Meloidogyne* spp. and Reniform nematode, *Rotylenchulus reniformis*

- There is not normal growth, the leaves become hard and brittle
- The fields look in patches because of abnormal growth due to distribution of nematodes.
- The yield is reduced finally.
- Heavily infested plants have a much reduced root system with large elongated galls in root-knot infested plants.
- Small galls along feeder roots and large galls on the main roots, are found in root knot infested plants.
- Rabie [112] reported that the combination of root-knot nematodes and other stress factors, such as drought and cold temperatures, are responsible in inflicting symptoms of 'False Panama Disease,' which is similar to 'Panama Disease'.
- In the former case transverse sections of rhizomes of infected plants showed reddish brown to brownish purple discoloured vascular tissue while in later case, reddish brown ring like symptoms were observed in cross section of pseudostem
- Leaf symptoms include progressive dying back of older leaves, starting at the tips. Galls occur on the primary and secondary roots, whilst distortion of roots and sometimes bifurcation occurs after heavy nematode infections.

4.3.2 Management

- Planting stocks which should be certified and free of infection.
- Application of carbofuran 3G @ 60 g/vine helps to improve the plant growth.
- Application of *P. fluorescens* @ 50 g/vine as biocontrol agent.
- Application of neem cake, vermicompost and pressmud, coriander intercrop and marigold intercrop in banana resulted in a general reduction in the population of plant parasitic nematodes. The nematicidal effect of neem cake, vermicompost, pressmud, marigold and coriander have been reported earlier [113–115].

4.3.3 *Helicotylenchus* spp. (Spiral nematodes)

Spiral nematodes are one of the most common group of nematodes infesting banana which include *H. multicinctus* (Cobb, 1893) Golden, 1956, *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961, and *Helicotylenchus erythrinae* (Zimmermann, 1904) Golden, 1956 [103, 106]. A survey of commercial banana plantations showed that species of *Helicotylenchus* (mainly *H. multicinctus*) were present in 95% of all the samples which had highest overall average [106]. These results were similar to previous studies. *H. multicinctus* was recorded in most of the soil and root samples collected in a survey [116]. Gowen and Quénéhervé [101] suggested that

H. multicinctus is often the major parasitic nematode on banana where temperature and rainfall conditions are suboptimal for the crop.

4.3.4 Damage

The above-ground symptoms are not specific and resemble damage caused by other nematode pests of banana. Symptoms of damage inflicted by *H. multicinctus* include discolouration of the root epidermis where small reddish lesions can be observed in the superficial cortical region also in rhizome tissue of infected plants along with reduction in the number of lateral roots [103]. Under severe infestations, the smaller lesions enlarge and coalesce leading to extensive necrosis in the outer cortical region of root and even root dieback sets in [117]. Toppling of plants can be observed due to poor anchorage under severe infestation [105]. No distinct biotypes or races have been reported in *H. multicinctus* [101].

4.3.5 Host range

H. multicinctus hosts range from most edible banana and plantain to various alternative host plants, such as pigweed (*Amaranthus* spp.), purslane (*Portulaca oleracea*) and ornamentals [101].

4.3.6 Nature of damage

Most damaging spiral nematode species spend most of their life cycle in root tissues of banana. Their buccal cavity is equipped with a hollow stylet which helps in puncturing and feeding the inner contents of cells. They multiply and build their population as high as a million in corm and root tissues and they alter the physical and functional integrity of the tissues. Nematodes disrupt nutrient and water uptake results in delay of growth and finally banana plants topple down. They destruct the primary roots so poor anchorage develops and that results in toppling of the plants.

4.3.7 Loss

In banana, the majority of the losses caused by *R. similis* infection is due to mass destruction of primary roots and could be present throughout entire root system, including the rhizome leading to poor anchorage [118]. During windy conditions, the plants with bunches often topple off due to heavy weight and poor anchorage, Hence the name “Toppling disease”. The nematodes move laterally in cortical region and colonize the cavities caused by parasitic and saprophytic fungi which results in greater lesion formation thus, leading to indirect disruption of stele which otherwise is rare. When this occurs, the entire root beyond the initial nematode entry site becomes functionless [103].

4.3.8 Host range

Duchame and Birchfield [119] established the existence of a *R. similis* biotype that also attacked citrus, it was confirmed that the *R. similis* did not attack citrus [120, 121]. An experiment to determine the host status was conducted to study the reaction of banana plants which were planted in *R. similis* infested soil. Out of 100 plants tested, only 20 were found to be able to act as host for *R. similis* [120, 121]. However, in field conditions, no record of *R. similis* was found to be associated with banana in both the National Collection of Nematodes (NCN) and South African Plant Parasitic Nematode Survey (SAPPNS) records.

4.4 Lesion nematodes (*Pratylenchus* spp.)

The lesion nematodes which are well represented in South African region, have a limited distribution in banana plantations. Two species of lesion nematodes which are most frequently found in South Africa plantations include *Pratylenchus coffeae* (Zimmerman, 1898) Filipjev and Schuurmans Stekhoven, 1941 and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 with the former one being more frequently encountered [116]. *Pratylenchus coffeae* was very effective in limiting the spread of *R. similis* in local banana-growing areas as the combination of legislative measures with the development of tissue culture based propagative material for producing nematode free plants, limited the spread of *R. similis* and *P. coffeae*. Tissue culture plants are developed in the laboratory using healthy planting material are free of pests and diseases. Before these plants are distributed to the producers, they have to be tested and declared virus free which is generally found in 25.9% of root and soil samples in commercial plantations [106]. Despite, these pests are widely distributed throughout the banana-producing areas, samples from several individual plantations had no lesion nematodes. The lesion nematode population in commercial banana plantations, varied from 0 to 1400 nematodes in 30 g/root. A survey was conducted in rural areas producing banana had shown that lesion nematodes were present in all areas where *P. coffeae* constituted only 3.2% of the nematode pest complex present in banana roots and 7.6% in soil samples [116].

4.5 Damage

The symptoms of damage caused by *P. coffeae* are very similar to those caused by *R. similis* as both are migratory endoparasites. They cause stunting of the plants, slow growth in vegetative phase, reduced number of leaves, lower bunch weight and reduce life span of plantations. There was reports of *P. coffeae* infections on banana plantations [107] which rendered the whole plantations unproductive.

4.5.1 Host range

The grapevine, citrus and veld (dune thicket, grasses) are the well-known hosts of *Pratylenchus coffeae* although it has a wide host range including many broad leaf weed species [101, 102] and information from both NCN and SAPPNS databases).

4.6 Management strategies

4.6.1 Legislation

In South Africa, when *R. similis* was discovered in banana under severe infection, the Government passed a legislation to prevent the spread of this nematode in new areas. This legislation is named as, “The Agricultural Pest Act 36 of 1983 [122]”. According to this legislation, for the transport of planting material from one area to another area, a permit is required during transportation. In this act, the propagation materials are identified as suckers, rhizomes and setts. The tissue culture plants are the best technique to get rid of this nematode, because these plants are healthy and do not carry any plant pathogens but eventhough, a permit is required when any nursery is established there.

4.6.2 Preparation of plant material

In most developed countries, nematode free banana planting material is exclusively produced from tissue culture-based methods for commercial purposes.

However, in underdeveloped countries particularly in rural areas, suckers and rhizomes are still being used to produce propagative material especially where tissue culture-based methods are lacking. This results in spread of plant parasitic nematodes to healthy soil from infected sites. In such cases, paring is recommended where visible lesions caused by nematode pests and banana weevil is removed so that only white rhizome tissue remains. In many African countries especially in South Africa paring is generally followed by hot water treatment for a specific period of time to kill remaining nematode population and found to be effective in managing nematode pests. Once the rhizomes are treated, they must be planted in nematode free soil and for that the soil should be tested in nematological laboratory. This could be obtained by using one or combinations of the following strategies, like, keeping the field fallow for a certain period, organic amendments, soil sterilisation through heat by using transparent plastic cover for several weeks or planting the suckers in virgin soil. The soil sterilisation is not that much feasible in a large area as the use of polythene to a large area is not practical because of high input cost.

4.6.3 Cultural control

Cultural methods are the cheap and easily followed method and also eco friendly. These methods include, fallowing for at least six months, is very useful [123, 124] as this is generally used as monocropping and because of this practice, nematode population build up and cannot be controlled through any method. Another method under this is, crop rotation with selected cover crops before banana cultivation that helps in reducing the nematode population so that the new suckers will not get the infection from very beginning like, Milne and Keetch [121] tested several cover crops and reported that radish (*Raphanus sativus*) and *Tagetes patula* reduced populations of *R. similis* after 5 months compared to that of ethylene dibromide (EDB) fumigation. Rotation with Buffalo grass (*Megathyrsus maximus* var. *trichoglum*; syn *Panicum maximum*) and purple bean (*Phaseolus atropurpureus*) also reported to control *R. similis* and *Meloidogyne* spp. Sugarcane crop (*Saccharum* hybrid) eliminated *R. similis* after 10 weeks [118]. The third method is intercropping. Intercropping is the cultivation of some particular crops with the main crops to manage the nematodes. These crops may be coffee (*Coffea arabica*), vegetables, maize (*Zea mays*) and cassava (*Manihot esculenta*). The next method is incorporation of organic manures in large volume. These manures after decomposition, release some phenolic compounds which are harmful for the nematode survival [125]. Application of 15 tons chicken manure/ha or 30 tons cattle manure/ha is generally recommended before planting banana [118]. The organic amendments have multiple beneficial effects like, increase in plant growth by improving soil structure and fertility, improvement in plant resistance and the stimulation of micro-organisms, which act as natural enemies of nematodes [125]. In cases of severe nematode infections, treatment of banana plants with a nematicide is recommended.

4.7 Clean propagative materials

4.7.1 Banana tissue culture

To prevent the spread of pests and disease, use of tissue culture banana planting material is one of the best methods to avoid the nematode infection. The tissue cultured propagating material is grown in such a media that it is free from any disease. So, this is an important practice to get rid of any pathogen or nematode. Using these materials, the spread of nematodes and other pathogens is controlled from diseased

field to healthy field. In Hawaii, most of the banana fields are infested with plant-parasitic nematodes [126], micro-propagation from disease free materials using sterile techniques, offers a good way to obtain nematode free planting materials.

4.7.2 Hot-water treatment

A hot water dip has been successfully used to control burrowing nematodes and root knot nematodes in anthurium and ginger, respectively. Although, various temperature-time combinations ranging from 5 min @ 50°C to 25 min @ 55°C are recommended by researchers across the world, CTAHR researchers recommend soaking of banana suckers at 50°C for 10 min for disinfestation.

4.7.3 Modified solarization

Soil solarization involves heating the soil using natural solar radiation beneath a transparent plastic sheet to reach lethal temperatures for soilborne pests. The method is effective against a range of soil inhabiting pests, pathogens and nematodes which live in the top 4 inches (10 cm) of soil. The nematodes in the deep layers escape from the lethal temperature attained by this method.

4.7.4 Biological control

Many biocontrol agents like, fungal and bacterial bioagents are beneficial for the management of nematode pests of banana. In 1998, Daneel et al. [127] has demonstrated the efficacy of the soil fungus, *Purpureocillium lilacinum* (syn *Paecilomyces lilacinus*) for the control of banana nematode pests including *R. similis* and *Meloidogyne* spp. This bioproduct was also responsible for reducing the period of growth from flowering to harvesting which is helpful in escaping the nematode problems. This product was used at a dosage rate of 2×10^9 spores/g in suspension at 2–4 g/mat, depending on the severity of nematode infestation [128]. It was registered for use in South Africa on banana. *P. lilacinus* is a common soil fungus used as biocontrol agents that has been isolated from many different habitats around the world. It acts as a facultative egg pathogen of sedentary nematodes and also an important option to control juvenile and adult burrowing nematodes in banana. Mendoza et al. [129] reported that this nematode antagonistic fungus may be used as an integrated approach to control plant parasitic nematodes of banana.

4.7.5 Corm destruction

Since the most damaging stages of nematodes spend a considerable amount of their life cycle inside roots, killing of banana plants using a non-selective herbicide i.e., Glyphosate simultaneously kills the obligate endoparasitic stages of the nematodes. Thus greatly improving the potential of successive fallow to lower the nematode populations without using nematicides [130].

4.7.6 Cover-cropping

Since banana is a perennial crop, and farmers take the benefit of ratoon crop also within the same planting cost, it is difficult to manage nematodes over a longer period of time because the endoparasites are nearly impossible to manage even through chemicals. In this condition, growing of cover crop may become an option for the management. These cover crops release some chemicals which are having allelopathic compounds that are deleterious for the nematodes. Allelopathy is a

biological phenomenon by which an organism produces one or more biochemicals that negatively affect the growth, survival, and reproduction of other organisms. The plants like, marigold (*Tagetes* spp.), sunhemp (*Crotalaria juncea*), rapeseed (*Brassica napus*, [131]), velvet bean (*Mucuna pruriens*, [132]), sorghum-sudan grass (*Sorghum bicolor* × *Sorghum arundinaceum* var. *Sudanense*, [133]), are reported as cover crops having allelopathy against plant parasitic nematodes. Among all these crops, marigold was found the best in banana cropping system and the allelopathy differs with different species of nematodes and marigold and also the soil temperature has the influence over it [134].

4.8 Production of healthy seedlings of banana

For the preparation of healthy banana suckers, a mixture of soil with biocontrol formulations and organic cakes can be prepared and used for hardening the seedlings. This mixture may include two kg each of *P. fluorescens* 1% W. P., *Trichoderma harzianum* 1% W. P. and *Purpureocillium lilacinus* 1% W. P. + five kg of carbofuran or phorate or 25 kg of neem cake or pongamia cake for preparing one ton of final mixture.

4.9 Chemical control

Conventionally, synthetic derived nematicides have been widely used for nematode control on banana. Although fumigants have been highly effective [135], such products are not used in banana production mainly due to high input costs and now these are banned also to be used in agriculture. The carbamates and the organophosphates are used regularly in the banana cultivation as pre and post application. These chemicals are used as granular or liquid formulations. During application, these are sprayed around the base of pseudostems or suckers. In South Africa, a chemical, Furfuraldehyde is registered for banana and it can be used when the population of nematodes is below economic damage level [128]. Because of unawareness and hidden mode of life cycle of plant parasitic nematodes, they are not given so much importance although they cause sufficient loss in the yield. Therefore, it is recommended that nematode samples are taken annually for nematode population estimation and that nematicides are only applied to reduce nematode pest populations likely to limit yield or cause long term yield decline. Although pre plant treatments such as soil fumigation with Telone II® (1,3-dichloropropene) are very effective in suppressing nematode populations, such treatments are short lived compared to the life of a banana plot. The following treatments should be done to manage the nematodes in banana:

1. Use of tissue cultured (*in vitro* produced) plants.
2. Rotation with alternative crops for minimum of 2 years.
3. Fallow in the absence of banana 'volunteers' for 10–12 months.
4. Selection of disease-free suckers.
5. Paring of diseased tissue from corms.
6. Immersing suckers in hot water for a particular time and period.
7. Flooding for 8 weeks after having destroyed previous banana crop.
8. Applying a nematicide to planting hole and in fill soil.

9. Regular spot applications with nematicides.

4.10 Practices that maintain productivity and vigour

1. Support plants with bamboo poles or with string or ropes to prevent plants toppling.
2. Regular application of mulches of grass, leaves or organic waste.
3. Grow cultivars with robust stature and wind tolerance and endophytes [136] including *Trichoderma viride* and non-pathogenic *F. oxysporum* [137, 138].

5. Conclusions

In banana cultivation, plant parasitic nematodes are not causing much damage or the loss and is not that much economical but there is yield reduction and the production cost increases very high when compared to the yield. Nowadays in banana cultivation, tissue culture propagation became a common practice, so, after planting in an orchard, there is rare chance of getting infection of plant parasitic nematodes in the first year. The cultivators also become very aware about the monitoring and testing the soils before planting and after planting every year. After getting tested from the designated authorities, they follow the recommendations given by them. Thus, these producers manage effectively the infestation of nematode problems. But, we have to be very careful and always be ready with some alternative management practices that could be followed if found any severe infection. The need for the management is more necessary in small holding farmers and growers because of their small holding size.

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